RESEARCH

Development of Near-Isogenic Peanut Lines with and without Resistance to the Peanut Root-Knot Nematode

C. Corley Holbrook,* Patricia Timper, Weibo Dong, Craig K. Kvien, and Albert K. Culbreath

ABSTRACT

Peanut (*Arachis hypogaea* L.) cultivars are available that have high resistance to the peanut root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood race 1] or tomato spotted wilt, caused by tomato spotted wilt *Tospovirus* (TSWV); however, no cultivars exist that have resistance to both pathogens. The objective of this research was to combine resistance to both pathogens in a single genotype. During the course of this research, we also had the opportunity to develop near-isogenic lines with and without nematode resistance. Such near-isogenic lines can be valuable research tools to obtain a better understanding of the interaction of nematodes with other pathogens of peanut. Breeding populations were developed by hybridizing the TSWV-resistant cultivar C-99R with the nematode-resistant cultivar COAN. Selection for nematode resistance was conducted using standard greenhouse screening techniques. Selection for TSWV resistance was conducted in the field with natural virus infection. A breeding line (C724-19-15) was selected that had high resistance to both pathogens. A near-isogenic line (C724-19-25) susceptible to the peanut root-knot nematode was also developed. Both breeding lines exhibited higher resistance to TSWV and higher yield than standard check cultivars when grown in fields with little or no nematode pressure.

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Abbreviations: TSWV, tomato spotted wilt virus.

The peanut root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood race 1] is found throughout the peanut (*Arachis hypogaea* L.) producing region of the United States and is a significant economic pathogen of peanut in Texas and the southeast (AL, FL, GA, and SC), where it reduces peanut yields by 3 to 15% annually (Dickson, 1998; Koenning et al., 1999; Minton and Baujard, 1990).

Peanut is also susceptible to several other soil-borne pathogens, and the peanut root-knot nematode has been shown to exacerbate some of these diseases. A positive interaction between nematode injury and severity of Cylindrocladium black rot (*Cylindrocladium crotalariae*) has been demonstrated (Diomande and Beute, 1981a,b; Diomande et al., 1981; Culbreath et al., 1992a). *Meloidogyne arenaria* has also been shown to increase the severity of preemergence damping-off and pod rot caused by *Pythium myriotylum* (Garcia and Mitchell, 1975) and white mold caused by *Sclerotium rolfsii* (Beute and Rodriguez-Kabana, 1979; Rodriguez-Kabana et al., 1977). There is also evidence that high populations of *M. arenaria* nullified the moderate resistance to *S. rolfsii* in the peanut cultivar

Published in Crop Sci. 48:194–198 (2008).
doi: 10.2135/cropsci2007.06.0313

Southern Runner (Culbreath et al., 1992b). Timper et al. (2004) demonstrated that infection of peanut by *M. arenaria* can lead to an increase in aflatoxin contamination of peanut kernels when the plants are subjected to drought stress during pod maturation.

Since 1985 tomato spotted wilt, caused by tomato spotted wilt virus (TSWV), has become a major problem in peanut-producing areas of the southern United States. The disease is now common in most peanut-growing areas, including Georgia, Florida, Alabama, Texas, and North Carolina, and has become the most important disease problem for many peanut growers (Culbreath et al., 1997a). Peanut cultivars are available that have resistance to either the peanut root-knot nematode or TSWV, but no cultivars are available that have resistance to both pathogens. The objective of this research was to incorporate resistance to both pathogens in a single genotype. During the course of this research, we also developed high-yielding, TSWV-resistant near-isogenic lines with and without nematode resistance. These lines should be valuable research tools to obtain a better understanding of the interaction of nematodes with other pathogens of peanut.

MATERIALS AND METHODS

The original population was developed by crossing C-99R (Gorbet and Shokes, 2002), a cultivar with moderate resistance to TSWV (Wells et al., 2002) with COAN (Simpson and Starr, 2001), a cultivar with near immunity to the peanut root-knot nematode. The population was advance to the F_4 using single seed descent. Individual F_4 plants were harvested.

A few seeds from each plant were used to evaluate the population for resistance to *M. arenaria* using the greenhouse screening technique described by Holbrook et al. (1983) with three replications. Plants were grown in steam-pasteurized loamy sand (850 g kg^{-1} sand, 110 g kg^{-1} silt, 40 g kg^{-1} clay) and were inoculated with 3500 eggs of *M. arenaria* race 1 (Gibbs isolate) that had been cultured alternatively on tomato (*Lycopersicon esculentum* Mill. cv. Rutgers) and peanut to reduce potential contamination from *M. incognita* (a parasite of tomato but not peanut). Nematode inoculum was prepared using the NaOCl method (Hussey and Barker, 1973) and applied 10 d after planting.

Approximately 70 d after inoculation, plants were uprooted and washed clean of soil. The roots were placed in 1000-mL beakers containing 300 mL of 0.05% (v/v) phloxine B solution for 3 to 5 min (Daykin and Hussey, 1985). Each plant was indexed for root galls and egg masses based on a scale of 0 to 5 (0 = no galls or no egg masses, 1 = 1–2, 2 = 3–10, 3 = 11–30, 4 = 31–100, and 5 = more than 100 galls or egg masses per root system) (Taylor and Sasser, 1978).

The remaining $F_{4:5}$ seeds were planted the following year in single replicate plots at the Gibbs Farm (Tifton loamy sand [fine-loamy, kaolinitic, thermic Plinthic Kandiudults]) in Tift County, GA. Plots consisted of two rows 80 cm apart and 3 m long. Final spotted wilt intensity was evaluated in each plot using a disease intensity rating that represents a combination of incidence and severity as described by Culbreath et al. (1997b). The number of 0.3-m portions of row containing severely stunted, chlorotic, wilted or dead plants was counted and converted to a percentage of row length for comparison of genotypes. A single plot was selected based on resistance to TSWV and visual observation of yield after digging. We harvested 30 individual plants from this plot because the nematode screening data indicated that this family might still be segregating for nematode resistance. These 30 lines were evaluated in additional greenhouse and field screens, and a nematode-resistant (C724-19-15) and a nematode-susceptible (C724-19-25) line were selected for more intensive study.

These two breeding lines and the nematode-resistant and nematode-susceptible check cultivars were tested for resistance to *M. arenaria* using the greenhouse technique described above with six replications. After the plants were indexed for root galls and egg masses, roots were blotted dry and weighed, and nematode eggs were collected with 1.0% (v/v) NaOCl and counted.

The same genotypes were also planted on 14 May 2004 and 28 Apr. 2005 in fields with little or no *M. arenaria* at the Gibbs Farm in Tift County, GA. Each test was planted in a randomized complete block design with two replications in 2004 and three replications in 2005. Plots consisted of two rows 80 cm apart and 4.6 m long. Entries were planted at 13 seeds m^{-1}. Plots were managed throughout the growing season by standard grower practices and were irrigated as needed. Spotted wilt intensity was evaluated in each plot using the disease intensity rating as previously described. Plots were dug on 25 Sept. 2004 and 9 Sept. 2005. The crop was picked the following day using a self-propelled small plot combine. Pods were dried with forced air (35°C) until kernel moisture reached about 8%.

The two isolines and the check genotypes were also tested in two fields that were heavily infested with *M. arenaria*. One field was at the Bowen Farm (Ocilla loamy coarse sand [loamy, siliceous, semiactive, thermic Aquic Arenic Paleudults]) in Tift County, GA. This test was planted on 11 May 2006, dug on 19 September, and picked on 20 September. The other field was at the Gibbs Farm. This test was planted on 24 May 2006 and dug and picked on 10 October. Each test was planted in a randomized complete block design with four replications. Plots consisted of two rows 80 cm apart and 4.6 m long. Entries were planted at 20 seeds m^{-1}. Immediately after digging, the roots from 10 randomly selected plants were visually rated for the amount of root galling using a 0 (no galling) to 10 (severe galling) scale. With the exception of no nematicide usage, plots were managed throughout the growing season by standard grower practices and were irrigated as needed.

All data were subjected to analysis of variance, and genotypic means were compared by Fisher's protected least significant difference. Unless otherwise stated, all differences referred to in the text were significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Root-gall index, egg-mass index, and eggs per gram of fresh root all clearly indicated that C724-19-15 was resistant to *M. arenaria*, whereas C724-19-25 was susceptible (Table 1). The level of resistance for C724-19-15 was similar to COAN and 'NemaTAM' (Simpson et al., 2003), the two nematode-resistant cultivars. Galling and egg production

Table 1. Root galling, egg-mass ratings, and *Meloidogyne arenaria* reproduction on selected peanut genotypes when tested in the greenhouse.

Genotype	Root-gall index†	Egg-mass index†	Eggs per gram fresh root
'Georgia Green'	4.3 ‡	3.7	8125
'C-99R'	4.2	3.2	3563
C724-19-25	4.2	3.0	5698
'COAN'	1.5	0.7	206
C724-19-15	1.3	0.5	134
'NemaTAM'	1.0	0.5	171
LSD ($P \leq 0.05$)	1.0	1.1	3071

†Root-gall and egg-mass index on 0 to 5 scale: 0, no galls or no egg-masses; 1, 1–2; 2, 3–10; 3, 11–30; 4, 31–100; 5, more than 100 galls or egg masses per root system.

‡Data are means of six replications.

Table 2. Pod yield of selected peanut genotypes in a field without *Meloidogyne arenaria* at Tifton, GA, in 2004 and 2005.

Genotype	Pod yield		
	2004	2005	Mean
	kg ha^{-1}		
C724-19-25	5376	4708	4931
C724-19-15	5310	4533	4792
'C-99R'	–	3709	–
'Georgia Green'	2203	2256	2232
'NemaTAM'	1109	838	999
'COAN'	517	1045	889
LSD ($P \leq 0.05$)	873	868	670†

†Genotype × year interaction effects were not significant ($P > 0.05$). Therefore, data from the 2 yr were pooled for genotype comparisons.

Table 3. Final intensity of tomato spotted wilt of selected peanut genotypes at Tifton, GA, in 2004 and 2005.

Entry	Final intensity rating†		
	2004	2005	Mean
	%		
'COAN'	83	59	67
'NemaTAM'	67	53	62
'Georgia Green'	42	45	44
'C-99R'	–	36	–
C724-19-25	4	23	19
C724-19-15	4	16	11
LSD ($P \leq 0.05$)	18‡	12‡	

†Percentage of the total row length with plants severely affected by tomato spotted wilt.

‡Genotype × year interaction effects were significant ($P \leq 0.05$). Therefore, data were analyzed independently for each year.

for C724-19-25 were significantly higher than the nematode-resistant cultivars, and similar to the susceptible cultivars, Georgia Green (Branch, 1996) and C-99R.

In fields with little to no nematode pressure, COAN and NemaTAM exhibited yields that were significantly lower than Georgia Green (Table 2). Similar results were observed for COAN in a previous study (Holbrook et al., 2003). Although NemaTAM was shown to have a higher yield potential than COAN in Texas (Church et al., 2000), the yield difference in our test was not significant. COAN and NemaTAM are not commercially viable cultivars for the southeastern United States because of their high susceptibility to TSWV (Table 3). Both C724-19-15 and C724-19-25 exhibited significantly higher resistance to TSWV than these cultivars in both years of testing. The two breeding lines also exhibited higher resistance to TSWV than Georgia Green and C-99R, two cultivars that have been shown to have moderate levels of resistance to TSWV (Culbreath et al., 1996; Wells et al., 2002).

The yields for both the nematode-resistant and susceptible breeding lines were significantly higher than Georgia Green when tested in fields with little to no nematode pressure (Table 2). Although a previous study had documented competitive yields in breeding lines with moderate resistance to nematodes (Holbrook et al., 2003), this is the first report of competitive pod yield for a peanut genotype with a high level of nematode resistance when grown under severe pressure from TSWV.

In the southeastern United States, peanuts in fields infested with *M. arenaria* also experience pressure from TSWV. In such a situation, the yield of currently available virus-resistant cultivars will be reduced by nematode pressure, and the yield of currently available nematode-resistant cultivars will be reduced by TSWV. Because of its high level of resistance to both TSWV and *M. arenaria*, the breeding line C724-19-15 had significantly higher yield than all other entries when grown in two locations with high pressure from both pathogens (Table 4). Root-gall indices for C724-19-15 demonstrated a level of nematode resistance similar to NemaTAM and the nematode-resistant germplasm lines NR0812 and NR0817 (Anderson et al., 2006), in combination with a superior level of resistance to TSWV.

Meloidogyne arenaria is an important pathogen in several disease complexes of peanut (Beute and Rodriguez-Kabana, 1979; Culbreath et al., 1992a; Diomande et al., 1981; Garcia and Mitchell, 1975; Timper et al., 2004). It would be useful to have peanut genotypes with nematode resistance to use in studies to better understand the role of nematodes in these disease complexes. Previously, the only nematode resistant cultivars were COAN and NemaTAM, and results from these types of studies could be greatly confounded by their extreme susceptibility to TSWV. The nematode-resistant and nematode-susceptible near-isogenic lines that we have developed should be highly useful experimental tools since they have similar yields and a similar level of resistance to TSWV.

This is the first report of a high-yielding breeding line (C724-19-15) with excellent resistance to both the peanut root-knot nematode and TSWV. This breeding line should be valuable for peanut growers who have to deal with both pathogens. This is also the first report of high-yielding, TSWV-resistant near-isogenic lines with

Table 4. Mean yield and final intensity of tomato spotted wilt of selected peanut genotypes when grown at two Georgia locations heavily infested with *Meloidogyne arenaria* in 2006.

Genotype	Yield			Gall Index†			TSWV‡
	Gibbs	Bowen	Mean	Gibbs	Bowen	Mean	Gibbs
	kg/ha^{-1}						%
C724-19-15	5865	3054	4661‡	0.1	0.0	0.0§	3.8
C724-19-25	4794	1649	3446	7.1	6.8	7.0	11.3
C209-6-13	4017	1835	3082	4.4	4.3	4.3	5.0
NR0812	3519	1474	2837	1.0	1.7	1.3	16.7
NR0817	3424	1407	2560	2.9	1.0	1.9	10.8
'Georgia Green'	3334	1080	2583	6.1	5.2	5.6	20.0
'NemaTAM'	2215	1303	1824	0.3	0.7	1.4	30.8
LSD ($P \leq 0.05$)	710	287	533	2.5	2.3	1.9	4.5

†Gall index on a 0 (no galling) to 10 (severe galling) scale.

‡TSWV, tomato spotted wilt virus.

§Genotype × year interaction effects were not significant ($P > 0.05$). Therefore, data from the 2 yr were pooled for genotype comparisons.

and without nematode resistance. These lines should be valuable experimental tools for studying the interaction of *M. arenaria* with other peanut pathogens. These lines also should be valuable germplasm for research on resistance mechanisms to *M. arenaria* and research on the resistance gene(s) to *M. arenaria*.

Acknowledgments

The contributions and technical support of David Clements, Jason Golden, Thomas Hilton, Vickie Hogan, Kathy Marchant, Dannie Mauldin, Kyle Montfort, Betty Tyler, and William Wilson are gratefully acknowledged. This work was supported in part by funds from the Georgia Peanut Commission.

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